JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1996, p. 2650–2653 Vol. 34, No. 11
0095-1137/96/$04.00+0

Evaluation of Chiron HIV-1/HIV-2 Recombinant Immunoblot Assay

RICHARD L. KLINE,[1,2] DENISE MCNAIRN,[2] MARK HOLODNIY,[3] LARRY MOLE,[3] DAN MARGOLIS,[3] WILLIAM BLATTNER,[4] AND THOMAS C. QUINN[1,2]*

Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases,[1] and Viral Epidemiology Section, National Cancer Institute,[4] Bethesda, Maryland; The Division of Infectious Diseases, Johns Hopkins Hospital, Baltimore, Maryland[2]; and AIDS Research Center, Veterans Affairs, Palo Alto Health Care System, Palo Alto, California[3]

Received 29 May 1996/Returned for modification 11 July 1996/Accepted 8 August 1996

In a study to determine the reliability, sensitivity, and specificity of the Chiron RIBA HIV-1/HIV-2 Strip Immunoblot Assay (RIBA HIV-1/2 SIA) for confirmation of human immunodeficiency virus type 1 (HIV-1) and HIV-2 antibodies, 1,263 serum samples from various populations in the United States, Caribbean, Africa, India, and Thailand were evaluated by RIBA HIV-1/2 SIA, and the results were compared with those obtained by an HIV-1 Western blot (immunoblot) assay. All sera were tested by HIV enzyme immunoassay, RIBA HIV-1/2 SIA, and Western blotting. Samples with discrepant results were further tested by an HIV-1 and/or HIV-2 immunofluorescent-antibody assay and HIV-1 p24 antigen assay. The RIBA HIV-1/2 SIA detected all 17 HIV-1 and HIV-2 dually reactive serum samples, all 215 HIV-2-positive serum samples, and 480 of 481 HIV-1-positive serum samples for a sensitivity of 99.8%. Of 548 negative samples, 523 were RIBA HIV-1/2 SIA negative, for a specificity of 95.4%, with 22 (4%) samples interpreted as indeterminate and 3 (0.6%) interpreted as falsely positive. Western blotting detected 391 of 548 negative samples (specificity, 71.4%), with 152 (27.7%) samples interpreted as indeterminate and 5 (0.9%) interpreted as falsely positive. In conclusion, the RIBA HIV-1/2 SIA had a sensitivity comparable to that of Western blotting and could discriminate HIV-1 from HIV-2 in one blot, providing a cost advantage. Because of its high degree of specificity, the RIBA HIV-1/2 SIA further reduced the number of indeterminate results found by Western blotting, providing a more accurate means of assessing seronegative individuals.

The current standard for serologically confirming infection with the human immunodeficiency virus (HIV) is the Western blot (immunoblot) assay. However, nonspecific reactivity on Western blots can occur with HIV-negative sera, leading to inconclusive or indeterminate results. The causes of indeterminate results, although not clearly understood, have been associated with contaminating cellular antigens on viral lysate-based blot strips, autoantibodies, pregnancy, elevated bilirubin levels, and in vitro hemolysis (4). Approximately 20 to 33% of HIV type 1 (HIV-1) enzyme immunoassay (EIA)-negative serum samples have indeterminate results by Western blotting (9, 15, 18). In addition, between 4 and 20% of serum samples that are repeatedly reactive by the HIV-1 EIA have indeterminate Western blot assay results (3, 6, 14, 17). The problems with indeterminate results are myriad. The suggested algorithm for an indeterminate result is to retest the patient after 2 to 3 months. The major problem is the anxiety created for the patient. Secondarily, considerable costs are associated with a second visit and repeat testing. Blood donations are discarded on the basis of repeatedly reactive screening test results, but indeterminate Western blot assay results place additional burdens on blood centers for donor notification and counseling and will prevent HIV-negative donors from reentering the donor pool. Individuals with indeterminate Western blot assay results may also have problems obtaining health insurance. From 1985 to 1990, as many as 69,000 blood donors tested indeterminate for HIV-1 by the Western blot assay, and their blood donations were discarded (11).

In 1986, HIV-2, a virus similar to but distinct from HIV-1, was isolated from Western Africa and was reported in patients with AIDS (2, 12). In 1987, the first case of HIV-2 infection in the United States was reported (5). In 1991, the first combination HIV-1/HIV-2 EIA was licensed, and in 1992, the U.S. Food and Drug Administration recommended that all blood donations be screened for both HIV-1 and HIV-2 infection. The HIV-2 genome shows approximately 60% homology to the HIV-1 genome in the *gag* and *pol* regions and 30 to 40% homology in the *env* region (10). Because of this genetic divergence, the Centers for Disease Control and Prevention and other investigators found that the sensitivities of HIV-1 EIAs for the detection of antibodies to HIV-2 ranged from 30 to 90% and that HIV-1 Western blot assays may be positive, indeterminate, or even negative when used to test HIV-2-positive sera (1, 7). Consequently, an HIV-2 enzyme-linked immunosorbent assay was developed, and it was recommended for use in testing EIA-positive samples that were negative or indeterminate by HIV-1 Western blot assays. At the time of this writing, this is the only test licensed by the U.S. Food and Drug Administration for the detection and confirmation of antibodies to HIV-2.

In order to improve the algorithm for confirming HIV-1 and HIV-2 infections, we tested an alternative to the Western blot assay, the Chiron RIBA HIV-1/HIV-2 Strip Immunoblot Assay (RIBA HIV-1/2 SIA), using blood specimens from various high-risk populations originating from different geographical regions. The sensitivity and specificity of the RIBA HIV-1/2 SIA were compared with the results of the Western blot assay, and the ability of the RIBA HIV-1/2 SIA to discriminate between HIV-1 and HIV-2 infection was evaluated.

* Corresponding author. Mailing address: Division of Infectious Diseases, The Johns Hopkins University School of Medicine, 1159 Ross Research Bldg., 720 Rutland Ave., Baltimore, MD 21205-2196. Phone: (410)-955-7635. Fax: (410)-955-7889.

TABLE 1. Comparison of RIBA HIV-1/2 SIA and Western blot assay

Sample	No. of samples							
	RIBA HIV-1/2 SIA					HIV-1/HIV-2 Western blot		
	Dually positive	HIV-1 positive	HIV-2 positive	Indeterminate	Negative	Positive	Indeterminate	Negative
HIV-1 and HIV-2 positive (n = 17)	17	0	0	0	0	16	1	0
HIV-1 positive (n = 481)	0	480	0	1	0	481	0	0
HIV-2 positive (n = 215)	0	0	215	0	0	158	57	0
Negative (n = 548)	0	2	1	22	523	5	152	391

MATERIALS AND METHODS

Sera or plasma from 1,263 HIV-infected or individual at high risk of HIV infection from geographically diverse areas were used to evaluate the RIBA HIV-1/2 SIA. In the present study, RIBA HIV-1/2 SIA testing was done regardless of the screening EIA result; many of these specimens were EIA nonreactive and would normally not be tested by a supplemental assay. The testing was performed in three different laboratories. The populations included HIV-infected patients and high-risk patients from the United States (n = 561); high-risk patients from Nigeria, Guinea Bissau, Ghana, and Cape Verde, where HIV-1 and HIV-2 infections are endemic (n = 369); patients from India and Thailand, where HIV-1 or HIV-2, or both, are rapidly spreading (n = 153); and patients from Zaire, Tanzania, Jamaica, and Trinidad, where HIV-1 is endemic (n = 100). Samples which contained antibodies to other viruses including human T-lymphotropic virus type 1, hepatitis C virus, herpes simplex virus, and hepatitis B virus surface antigen (n = 80) were also tested.

The RIBA HIV-1/2 SIA uses an immunoblot EIA technique for the detection of antibodies to HIV-1 and HIV-2. Each strip uses four recombinant viral antigens, p24 (*gag*), gp41 (*env*), gp120 (*env*), and p31 (*pol*), from HIV-1 and one recombinant viral antigen, p26 (*gag*), and a synthetic peptide derived from the HIV-2 transmembrane envelope protein. These antigens are immobilized on nitrocellulose strips as five bands (p24 and p26 are combined as one band). Each strip also has two different concentrations of an immunoglobulin G (IgG) control located at opposite ends of the strip. These internal controls are used to determine the intensities of the viral bands. Briefly, the RIBA HIV-1/2 SIA procedure is as follows: 20 μl of sample or control is added to a tube containing the RIBA HIV-1/2 SIA strip and specimen diluent. The tube is placed in a rack (which is supplied with the assay kit) and rocked at 16 to 20 cycles per min for 4 to 4.5 h at room temperature. The liquid in the tube is aspirated, and 1 ml of specimen diluent is added to the tube, which is rocked again for 30 to 35 min at room temperature. The liquid is again aspirated, 1 ml of wash buffer is added to the tube, the strip and wash buffer are transferred to a wash vessel (which is supplied with the assay kit), and the strip is washed twice. One milliliter of peroxidase-labeled goat anti-human IgG conjugate per strip is added to the wash vessel, and the vessel is rotated on a rotary shaker (110 ± 5 rpm) for 9 to 11 min at room temperature. The strips are washed three times, and 1 ml of working substrate (4-chloro-1-naphthol in phosphate-buffered hydrogen peroxide) per strip is added to the wash vessel, which is rotated for 15 to 20 min at room temperature. The strips are then washed two times in deionized water and allowed to dry on absorbent paper in the dark for 30 min, and the results are interpreted within 3 h.

The RIBA HIV-1/2 SIA result is scored by reading the intensity of the viral bands as follows: − (absence of band), ± (less than level I control), 1+ (equal to level I IgG control, 2+ (greater than level I IgG control), 3+ (equal to level II IgG control), and 4+ (greater than level II IgG control). The criteria for the interpretation of the RIBA HIV-1/2 SIA result is based on the presence of viral bands and their scores, as follows. For a band pattern of no bands with greater than ± reactivity, the result is negative. For band patterns of 1+ or greater reactivity to the gp41 band and any other HIV-1 band and <1+ reactivity to the HIV-2 envelope peptide band, the result is HIV-1 positive. For band patterns of 1+ or greater reactivity to the HIV-2 envelope peptide band and any other HIV-1 band and <1+ reactivity to the gp120 band, the result is HIV-2 positive. For band patterns of 1+ or greater reactivity to the HIV-2 envelope peptide band the gp120 band, the p31 band, and/or the p24/26 band and <1+ reactivity to the gp41 band, the result is HIV-2 positive. For band patterns of 1+ or greater reactivity to the HIV-1 gp41 band, the gp120 band, and the HIV-2 envelope peptide bands, the result is HIV-1 and HIV-2 positive. For band patterns of 1+ or greater reactivity to any HIV bands, but the pattern does not meet the criteria for HIV-1 positive or HIV-2 positive, the result is indeterminate.

All sera were tested for HIV-1 and HIV-2 infection by EIA (Abbott Laboratories, Abbott Park, Ill.; Organon Teknika, Durham, N.C.), RIBA HIV-1/2 SIA, and a Western blot assay (Cambridge Biotech, Worcester, Mass.; Diagnostic Biotechnology Ltd.) All tests were run as specified by the manufacturers. A Western blot assay result was interpreted as positive if two of the following three bands were present: p24, gp41, and gp120/gp160. A Western blot assay result was interpreted as negative if no virus-specific bands were present. If virus-specific bands were present but they did not meet the criteria for a positive Western blot assay result given above, the result for the blot was considered indeterminate. Any samples with discrepant results between RIBA HIV-1/2 SIA and Western blotting were further tested by the Waldheim Pharmazeutika Fluorognost HIV-1 immunofluorescent-antibody assay (IFA) and HIVAG EIA (p24 antigen assay; Abbott Laboratories). Abbott HIVAG testing was done to determine if specimens with discrepant results were from patients who were early seroconverters. Specimens with discrepant HIV-2 results were further tested by an HIV-2 EIA (Genetic Systems Corp, Redmond, Wash.) and the Fluorognost HIV-2 IFA (which is available for research use only). The sensitivity and specificity of the RIBA HIV-1/2 SIA were calculated against the results of the Western blot assay. For specimens with discrepant results, the results of IFA and the p24 antigen assay were used to resolve the results for specimens with discrepant results and assign a most likely HIV status. Even after evaluating all the tests results, the results for some samples were still inconclusive since they were indeterminate by all tests. Consequently, data for those samples were excluded from analysis.

RESULTS

Of the 1,263 blood samples, Western blot assay, IFA, and the p24 antigen assay detected 481 HIV-1-positive specimens, 215 HIV-2-positive specimens, 17 dually reactive specimens, 548 negative specimens, and 2 indeterminate specimens. By the RIBA HIV-1/2 SIA, all 17 dually reactive samples were interpreted as dually reactive for HIV-1 and HIV-2, 480 of 481 HIV-1-positive samples were HIV-1 RIBA positive, and all HIV-2 positive samples were HIV-2 RIBA positive, for an overall sensitivity of 99.8% (Table 1). All HIV-1-positive samples and 16 of 17 dually reactive samples were HIV-1 Western blot assay positive, for a sensitivity of 99.8%. Of the 548 negative samples, 523 were interpreted as RIBA HIV-1/2 SIA negative, 22 negative samples were interpreted as RIBA HIV-1/2 SIA indeterminate, 2 negative samples were interpreted as HIV-1 positive, and 1 negative sample was interpreted as HIV-2 positive, for a specificity of 95.4%. All RIBA HIV-1/2 SIA-indeterminate or false-positive samples were HIV-1/HIV-2 IFA negative, and none of the specimens tested in the present trial were positive by the HIVAG EIA. For the HIV-1 Western blot assay, 391 of 548 negative samples were interpreted as negative, for a specificity of 71.4%, and 152 negative samples, 57 HIV-2-positive samples, and 1 dually reactive sample were interpreted as indeterminate. Five negative samples were interpreted as HIV-1 positive.

The data were analyzed by population, and the sensitivity, specificity, and percent indeterminate for the RIBA HIV-1/2 SIA and the Western blot assay are provided for each group (Table 2). In all populations, the specificity of the RIBA HIV-1/2 SIA was greater than the specificity of the Western blot assay, and the number of RIBA HIV-1/2 SIA-indeterminate specimens was less than the number of Western blot assay-indeterminate specimens. In addition, infection with other pathogens including human T-lymphotropic virus type 1, herpes simplex virus, hepatitis C virus, and hepatitis B virus did not affect the specificity of the RIBA HIV-1/2 SIA or the Western blot assay (Table 2).

TABLE 2. Sensitivity, specificity, and percent indeterminate samples by RIBA HIV-1/2 SIA and Western blot assay by population

Population	No. of subjects	Sensitivity (%)		Specificity (%)		% Indeterminate	
		RIBA SIA	Western blot	RIBA SIA	Western blot	RIBA SIA	Western blot
AIDS	200	100	100			0	0
Other diseases	80	93.8	100	96.9	79.2	3.8	13.8
High risk	361	100	100	98.1	76.8	1.8	20.5
Subjects from areas where HIV-1 is endemic	149	100	100	85.0	30.0	2.0	9.4
Subjects from areas where HIV-1 and HIV-2 are endemic	471	100	99.2[a]	89.4	58.3	3.0	23.6

[a] Sensitivity for this population from areas where HIV-1/HIV-2 are endemic was calculated by using only HIV-1-positive and both HIV-1- and HIV-2-positive samples because Western blotting is not expected to detect HIV-2-positive samples.

DISCUSSION

In the analysis of 1,263 serum samples from individuals from geographically diverse areas, the RIBA HIV-1/2 SIA proved to be a reliable alternative to the Western blot assay. The RIBA HIV-1/2 SIA had a sensitivity of 99.8%, which is comparable to that of the Western blot assay in our study, and correctly detected all 17 dually reactive samples, 480 of 481 HIV-1-positive samples, and 215 HIV-2-positive samples. The assay was easy to perform, taking approximately 6 h for completion, compared with 16 to 20 h for the licensed Western blot assay used in the study. The bands on the RIBA HIV-1/2 SIA were distinct and easy to see. Although some subjective interpretation is required when scoring the bands on the RIBA HIV-1/2 SIA, as with Western blot assay interpretation, the use of IgG internal controls simplified the process. Unlike the Western blot assay, there were no nonviral bands on the RIBA HIV-1/2 SIA which could affect interpretation of the results for the sample.

Indeterminate results have always been a major problem with Western blots. One potential cause of indeterminate results may be that the samples were obtained from patients in the acute phase of HIV infection, when antibody production to HIV has just begun and antibodies to each of the major gene products are not yet present. Studies done with samples with indeterminate Western blot assay results, including the present study, have shown that this occurs extremely rare (13, 15, 16). In the present study, none of the specimens indeterminate by Western blotting were positive for p24 antigen. The second and main cause of indeterminate Western blot assay results are samples that are falsely reactive because of contaminating cellular antigens, autoantibodies, pregnancy, elevated bilirubin levels, and in vitro hemolysis (4). In the present study, 210 (16.7%) samples were indeterminate by the Western blot assay; 57 were from HIV-2-positive subjects, 152 were from HIV-1- and HIV-2-negative subjects, and 1 was from a dually reactive subject. In contrast, the RIBA HIV-1/2 SIA had fewer indeterminate results ($n = 22$) and false-positive results ($n = 3$) compared with the Western blot assay ($n = 210$ and 5, respectively), and thereby had a higher specificity (95.4%) compared with that of the Western blot assay (71.4%). If data for samples indeterminate by the RIBA HIV-1/2 SIA and Western blotting are excluded from analysis, the specificity of the RIBA HIV-1/2 SIA was 99.4% and that of Western blotting was 98.7%. Since the present study was a direct comparison of the RIBA HIV-1/2 SIA and the Western blot assay, the frequency of indeterminate results would probably have been lower if only specimens that were repeatedly reactive by EIA were analyzed.

The lower frequency of indeterminate results with the RIBA HIV-1/2 SIA does avoid supplemental and repeat testing and can help prevent patient anxiety over inconclusive results inherent from indeterminate Western blot assay results. However, it is disturbing that three samples tested false positive by the RIBA HIV-1/2 SIA. Because these specimens were not retested by the RIBA HIV-1/2 SIA because of insufficient volume, it is not known whether these results are inherent in the assay or are due to operator error or specimen mix-up. Although the rate of false-positive results by the RIBA HIV-1/2 SIA was lower than that by the Western blot assay ($n = 5$), false-positive results are of even greater concern than indeterminate results, and every effort should be made to improve the assay or procedure to completely eliminate false-positive results.

The RIBA HIV-1/2 SIA proved to be excellent in its ability to discriminate HIV-1 from HIV-2 infection. The ability to differentiate HIV-1 from HIV-2 may be helpful in a clinical setting. There is some inference that the pathogenesis of HIV-2 is different from that of HIV-1 and that HIV-2 may have a longer latent period (8). As treatment advances occur, treatment protocols for HIV-2 may be different from those for HIV-1, so the need to ascertain which virus is responsible for the infection may be important. The ability to differentiate HIV-1 from HIV-2 would also be important in epidemiological and natural history studies when both viruses are present. The RIBA HIV-1/2 SIA could also be used as part of a more cost-efficient diagnostic algorithm, since antibodies to both viruses can be differentiated by only one test. In contrast to performing two Western blot assays to identify HIV-1- and HIV-2-positive samples, a single RIBA HIV-1/2 SIA would lower costs and save technician time. Unfortunately, the cost of the assay as well as that of the Western blot assay still remain too high to be used routinely in resource-poor areas.

REFERENCES

1. **Busch, M. P., L. Petersen, C. Schable, and H. Perkins.** 1990. Monitoring blood donors for HIV-2 infection by testing anti-HIV-1 reactive sera. Transfusion **30:**184–187.
2. **Cabrian, K., K. Shriver, L. Goldstein, and M. Krieger.** 1988. Human immunodeficiency virus type 2 (HIV-2) infection. Transfusion **28:**192–195.
3. **Carlson, J. R., M. L. Bryant, S. H. Hinrichs, J. K. Yamamoto, N. B. Levy, J. Yee, J. Higgins, A. M. Levine, P. Holland, M. B. Gardner, and N. C. Pedersen.** 1985. AIDS serology testing in low- and high-risk groups. JAMA **259:**2574–2579.
4. **Celum, C. L., R. W. Coombs, M. Jones, V. Murphy, L. Fisher, C. Grant, L. Corey, T. Inui, M. H. Wener, and K. K. Holmes.** 1994. Risk factors for repeatedly reactive HIV-1 EIA and indeterminate Western blots. A population-based case-control study. Arch. Intern. Med. **154:**1129–1137.
5. **Centers for Disease Control.** 1988. AIDS due to HIV-2 infection in New Jersey. Morbid. Mortal. Weekly Rep. **37:**33–35.
6. **Centers for Disease Control.** 1989. Interpretation and use of the Western blot assay for serodiagnosis of human immunodeficiency virus type 1 infections. Morbid. Mortal. Weekly Rep. **38:**1–7.
7. **Chattopadhya, D., S. Kumari, and T. Verghese.** 1991. Limited evidence of

human immunodeficiency virus type 2 (HIV-2) infection in sera from blood donors showing positive ELISA but negative or indeterminate Western blot reactivity for HIV-1 infection. J. Commun. Dis. **23:**206–207.

8. **Dufoort, G., A. Courouce, R. Ancelle-Park, and O. Bletry.** 1988. No clinical signs 14 years after HIV-2 transmission via blood transfusion. Lancet **ii:**521.
9. **Genesca, J., B. W. Jett, J. S. Epstein, J. Wai-Kuo Shih, I. K. Hewlett, and H. J. Alter.** 1989. What do Western blot indeterminate patterns for human immunodeficiency virus mean in EIA-negative blood donors? Lancet **ii:** 1023–1025.
10. **Guyader, M., M. Emerman, P. Sonigo, F. Clavel, L. Montagnier, and M. Alizon.** 1987. Genome organization and trans-activation of the human immunodeficiency virus type 2. Nature (London) **326:**662–669.
11. **Jackson, J. B., K. L. MacDonald, J. Cadwell, C. Sullivan, W. E. Kline, M. Hanson, K. J. Sannerud, S. L. Stramer, N. J. Fildes, S. Y. Kwok, J. J. Sninsky, R. J. Bowman, H. F. Polesky, H. H. Balfour, Jr., and M. T. Osterholm.** 1990. Absence of HIV infection in blood donors with indeterminate Western blot tests for antibody to HIV-1. N. Engl. J. Med. **322:**217–222.
12. **Kanki, P. J., F. Barin, S. M'Boup, J. S. Allan, J. L. Romet-Lemonne, R. Marlink, M. F. McLane, T. H. Lee, B. Arbeille, F. Denis, and M. Essex.** 1986. New human T-lymphotropic retrovirus related to simian T-lymphotropic virus type III. Science **232:**238–243.
13. **Knuver-Hopf, J., H. Heinze, B. Lambrecht, H. Mohr, J. Beyer, and H. Schmitt.** 1993. Blood donations indeterminate in HIV-1 Western blot analysed by IgM immunoblot and polymerase chain reaction. Vox Sang. **64:**89–93.
14. **MacDonald, K. L., J. B. Jackson, R. J. Bowman, H. F. Polesky, F. S. Rhame, H. H. Balfour, Jr., and M. T. Osterholm.** 1989. Performance characteristics of serological tests of human immunodeficiency virus type 1 (HIV-1) antibody among Minnesota blood donors. Ann. Intern. Med. **110:**617–621.
15. **Midthun, K., L. Garrison, M. L. Clements, H. Farzadegan, B. Fernie, and T. Quinn.** 1990. Frequency of indeterminate Western blot tests in healthy adults at low risk for human immunodeficiency virus infection. J. Infect. Dis. **162:**1379–1382.
16. **Phair, J., D. Hoover, J. Hupriker, R. Detels, R. Kaslow, C. Rinaldo, and A. Saah.** 1992. The significance of Western blot assays indeterminate for antibody to HIV in a cohort of homosexual/bisexual men. The Multicenter AIDS Cohort Study. J. Acquired Immune Defic. Syndr. **5:**988–992.
17. **Schwartz, J. S., P. E. Dans, and B. P. Kinosian.** 1988. Human immunodeficiency virus test evaluation, performance, and use. JAMA **259:**2574–2579.
18. **Westblom, T. U., R. B. Belshe, G. J. Gorse, E. L. Anderson, and C. F. Berry.** 1990. Characteristics of a population volunteering for human immunodeficiency virus immunization. Int. J. STD AIDS **1:**126–128.